LIQUIZYME

ALP Test Kit

(PNPP Method)

BEACON

INTENDED USE:

This reagent kit is intended for ***"in vitro"*** quantitative determination of Alkaline Phosphatase activity in serum/plasma.

CLINICAL SIGNIFICANCE:

Alkaline Phosphatase (ALP) is an enzyme of the Hydrolase class of enzymes and acts in an alkaline medium. It is found in high concentrations in the liver, biliary tract epithelium and in the bones. Normal levels are age dependent and increase during bone development. Increased levels are associated mainly with liver and bone disease. Moderate increases are seen in Hodgkins disease and congestive heart failure.

PRINCIPLE:

Alkaline Phosphatase (ALP) at an alkaline pH hydrolases p-Nitrophenylphosphate to form p-Nitrophenol and phosphate. The rate of formation of p-Nitrophenol is measured as an increase in absorbance which is proportional to the ALP activity in the sample.

REACTION:

$$\text{P-NPP} + H_2O \xrightarrow[\text{Alk. medium}]{\text{ALP}} \text{PNP} + PO_4$$

CONTENTS:

Reagent 1 - AMP Buffer
Reagent 2 - PNPP Substrate

MATERIALS REQUIRED BUT NOT PROVIDED:-

- Clean & Dry Glassware.
- Laboratory Glass Pipettes or Micropipettes & Tips.
- Bio-Chemistry Analyzer.

SAMPLES:

Unhaemolysed Serum, ALP is reported to be stable in serum for 3 days at 2 - 8°C.

PREPARATION OF REAGENT & STABILITY :

Working Reagent :
Mix 4 part of Reagent - 1 with 1 part of Reagent - 2
Stability : 5 Days at 20 -25°C .30 days at 2-8°C.

GENERAL SYSTEM PARAMETERS:

Reaction type	: Kinetic (Increasing)
Wave length	: 405 nm
Temperature	: 37°C
Delay	: 60 sec.
Interval	: 60 sec.
No.of reads	: 3
Reagent volume	: 1.0 ml
Sample volume	: 20 µl
Factor	: 2764
Zero setting	: Deionosed water
Light path	: 1 cm

PROCEDURE:

Pipette into clean dry test tube labelled as Test (T) :

Addition Sequence	(T)
Working Reagent	1.0 ml
Sample	20 µl

Mix well and read the initial absorbance A_0after 1 minute & repeat the absorbance reading after every 1, 2, & 3 minute. Calculate the mean absorbance change per minute (Δ A/min).

CALCULATION :

ALP activity (U/L) = ΔA/min. x 2764.

NORMAL VALUE : .

Children (3-15 yrs) : 104 - 390 U/L
Adults : 25 - 140 U/L
Each Laboratory should establish it's own normal range representing its patient population.

LINEARITY :

This procedure is linear upto 1600 U/L. If the absorbance change (ΔA/min.) Exceeds 0.570, dilute the sample with normal saline (NaCl 0.9%) and repeat the assayMultiply result by dilution factor.

QUALITY CONTROL :

For accuracy, it is advised to run known serum controls with each assay.

LIMITATION & PRECAUTIONS :

1. Storage conditions as mentioned on the kit to be adhered.
2. Do not freeze or expose the reagents to higher temperature as it may affect the performance of the kit.
3. Before the assay bring all the reagents to room temperature.
4. Avoid contamination of the reagent during assay process.
5. Use clean glassware free from dust or debris.
6. Reagent : sample ratio as mentioned here above must be strictly observed as any change in to it will adversly effect the factor.
7. Donot use the reagent if the reagent is hazy or cloudy.

BIBLIOGRAPHY :

1.Rec. GSCC (DGKC) ; J. Clin. Chem. Clin. Biochem. 1972; 10 : 182.
2.Heerspik. W., Hafkenscheidt J. C. M., Siepelvander Ven Jongekryg J., Djit C. C. M., Enzyme 25, 333 - 341 (1980).

CODE NO.	PACK SIZE	Reagent 1	Reagent 2
S05C	4 x 20 ml + 1 x 20 ml	4 x 20 ml	1 x 20 ml

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